(FILE 'HOME' ENTERED AT 12:52:00 ON 21 MAY 1999)

FILE 'CAPLUS, SCISEARCH, MEDLINE, BIOSIS, CANCERLIT, AGRICOLA' ENTERED AT 12:52:04 ON 21 MAY 1999

L1	116	S RUVKUN G/AU
L2	19	S L1 AND DAF
L3	10	DUP REM L2 (9 DUPLICATES REMOVED)
L4	1	S L3 AND PTEN
L5	3	S L1 AND PTEN
L6	1	DUP REM L5 (2 DUPLICATES REMOVED)
L7	1	S (GLUCOSE TOLERANCE) AND DAF
L8	3	S OBESITY AND DAF
L9	2	DUP REM L8 (1 DUPLICATE REMOVED)
L10	952	S PTEN
L11	455	S L10 AND PHOSPHATASE
L12	8	S L11 AND DAF-18
L13	3	DUP REM L12 (5 DUPLICATES REMOVED)
L14	16	S L11 AND ASSAY
L15	6	DUP REM L14 (10 DUPLICATES REMOVED)
L16	0	S TRANSGENIC AND L11
L17	0	S TRANSGENIC AND DAF-18

(FILE 'USPAT' ENTERED AT 12:38:43 ON 21 MAY 1999)

L1	0	S	RUVKUN ?/IN
L2	0	S	DAF-18
L3	0	S	S PTEN
L4	7	S	OBESITY AND DAF
L5	0	S	(GLUCOSE TOLERANCE) AND DAF
L6	0	S	LIPID PHOSPHATASE
L7	31	S	TRANSGENIC AND DAF
L8	0	S	RUVKUN G/IN
L9	12149	S	PHOSPHATASE
L10	2934	S	L9 AND LIPID
L11	0	S	L10 AND PTEN
L12	0	S	L10 AND MMAC
L13	0	S	L10 AND MMAC1
L14	0	S	L10 AND TEP1
L15	8	S	TEP1
L16	1	S	MMAC1

(FILE 'HOME' ENTERED AT 12:52:00 ON 21 MAY 1999)

FILE 'CAPLUS, SCISEARCH, MEDLINE, BIOSIS, CANCERLIT, AGRICOLA' ENTERED AT 12:52:04 ON 21 MAY 1999
L1 116 S RUVKUN G/AU
L2 19 S L1 AND DAF
L3 10 DUP REM L2 (9 DUPLICATES REMOVED)

=> d Ti so au ab L3 1-10

L3 ANSWER 1 OF 10 BIOSIS COPYRIGHT 1999 BIOSIS
TI **DAF**-16 regulates the transcription of the insulin-sensitive gene insulin-like growth factor binding protein (IGFBP)-1.
SO FASEB Journal, (March 12, 1999) Vol. 13, No. 4 PART 1, pp. A77. Meeting Info.: Annual Meeting of the Professional Research Scientists for Experimental Biology 99 Washington, D.C., USA April 17-21, 1999 ISSN: 0892-6638.
AU Alexander-Bridges, M.; Cahill, C.; Ogg, S.; **Ruvkun, G.**; Avruch, J.; Nasrin, N.; Tzivion, G.

L3 ANSWER 2 OF 10 SCISEARCH COPYRIGHT 1999 ISI (R)
TI Aging, life span, and senescence
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (15 SEP 1998) Vol. 95, No. 19, pp. 11034-11036. Publisher: NATL ACAD SCIENCES, 2101 CONSTITUTION AVE NW, WASHINGTON, DC 20418. ISSN: 0027-8424.
AU Guarente L (Reprint); **Ruvkun G**; Amasino R

L3 ANSWER 3 OF 10 SCISEARCH COPYRIGHT 1999 ISI (R) DUPLICATE 1
TI Caenorhabditis elegans Akt/PKB transduces insulin receptor-like signals from AGE-1 PI3 kinase to the **DAF**-16 transcription factor
SO GENES & DEVELOPMENT, (15 AUG 1998) Vol. 12, No. 16, pp. 2488-2498. Publisher: COLD SPRING HARBOR LAB PRESS, 1 BUNGTOWN RD, PLAINVIEW, NY 11724. ISSN: 0890-9369.
AU Paradis S; **Ruvkun G (Reprint)**
AB A neurosecretory pathway regulates a reversible developmental arrest and metabolic shift at the Caenorhabditis elegans dauer larval stage, Defects in an insulin-like signaling pathway cause arrest at the dauer stage. We show here that two C. elegans Akt/PKB homologs, akr-1 and akt-2, transduce insulin receptor-like signals that inhibit dauer arrest and that AKT-1 and AKT-2 signaling are indispensable for insulin receptor-like signaling in C. elegans, A loss-of-function mutation in the Pork head transcription factor **DAF**-16 relieves the requirement far Akt/PKB signaling, which indicates that AKT-1 and AKT-2 function primarily to antagonize **DAF**-16. This is the first evidence that the major target of Akt/PKB signaling is a transcription factor. An activating mutation in akt-1, revealed by a genetic screen, as well as increased dosage of wild-type akt-1 relieves the requirement for signaling from AGE-1 PI3K, which acts downstream of the **DAF**-2 insulin/IGF-1 receptor homolog. This demonstrates that Akt/PKB activity is not necessarily dependent on AGE-1 PI3K activity. akt-1 and akt-2 are expressed in overlapping patterns In the nervous system and in tissues that are remodeled during dauer formation.

L3 ANSWER 4 OF 10 SCISEARCH COPYRIGHT 1999 ISI (R) DUPLICATE 2
TI The C-elegans PTEN homolog, **DAF**-18, acts in the insulin receptor-like metabolic signaling pathway
SO MOLECULAR CELL, (DEC 1998) Vol. 2, No. 6, pp. 887-893. Publisher: CELL PRESS, 1050 MASSACHUSETTES AVE, CIRCULATION DEPT, CAMBRIDGE, MA 02138. ISSN: 1097-2765.
AU Ogg S; **Ruvkun G (Reprint)**
AB An insulin-like signaling pathway, From the **DAF**-2 receptor, the AGE-I phosphoinositide 3-kinase, and the AKT-1/AKT-2 serine/threonine kinases to the **DAF**-16 Fork head transcription factor, regulates the metabolism, development, and life span of Caenorhabditis elegans. Inhibition of **daf**-18 gene activity bypasses the normal requirement for AGE-I and partially bypasses the need for **DAF**-P signaling. The suppression of age-1 mutations by a **daf**-18 mutation depends an AKT-1/AKT-2 signaling, showing that **DAF**-18 acts between AGE-1 and the AKT input to **DAF**-16 transcriptional regulation. **daf**-18 encodes a homolog of the human turner

suppressor PTEN (MMAC1/TEP1), which has 3-phosphatase activity toward phosphatidylinositol ,5-trisphosphate (PIP3). **DAF**-18 PTEN may normally limit AKT-1 AKT-2 activation by decreasing PIP3, [redacted]els. The action of **daf**-18 in this metabolic control pathway suggests that mammalian PTEN may modulate insulin signaling and may be variant in diabetic: pedigrees.

L3 ANSWER 5 OF 10 SCISEARCH COPYRIGHT 1999 ISI (R) DUPLICATE 3
TI An insulin-like signaling pathway affects both longevity and reproduction in Caenorhabditis elegans
SO GENETICS, (FEB 1998) Vol. 148, No. 2, pp. 703-717.
Publisher: GENETICS, 428 EAST PRESTON ST, BALTIMORE, MD 21202.
ISSN: 0016-6731.
AU Tissenbaum H A; **Ruvkun G (Reprint)**
AB Mutations in **daf**-2 and age-1 cause a dramatic increase in longevity as well as developmental arrest at the dauer diapause stage in Caenorhabditis elegans. **daf**-2 and age-1 encode components of an insulin-like signaling pathway. Both **daf**-2 and age-1 act at a similar point in the genetic epistasis pathway for dauer arrest and longevity and regulate the activity of the **daf**-16 gene. Mutations in **daf**-16 cause a dauer-defective phenotype and are epistatic to the diapause arrest and life span extension phenotypes of **daf**-2 and age-1 mutants. Here we show that mutations in this pathway also affect fertility and embryonic development. Weak **daf**-2 alleles, and maternally rescued age-1 alleles that cause life span extension but do not arrest at the dauer stage, also reduce fertility and viability. We find that age-1(hx546) has reduced both maternal and zygotic age-1 activity. **daf**-16 mutations suppress all of the **daf**-2 and age-1 phenotypes, including dauer arrest, life span extension, reduced fertility, and viability defects. These data show that insulin signaling, mediated by **DAF**-2 through the AGE-1 phosphatidylinositol-3-OH kinase, regulates reproduction and embryonic development, as well as dauer diapause and life span, and that **DAF**-16 transduces these signals. The regulation of fertility, life span, and metabolism by an insulin-like signaling pathway is similar to the endocrine regulation of metabolism and fertility by mammalian insulin signaling.

L3 ANSWER 6 OF 10 SCISEARCH COPYRIGHT 1999 ISI (R) DUPLICATE 4
TI The **DAF**-3 Smad protein antagonizes TGF-beta-related receptor signaling in the Caenorhabditis elegans dauer pathway
SO GENES & DEVELOPMENT, (15 OCT 1997) Vol. 11, No. 20, pp. 2679-2690.
Publisher: COLD SPRING HARBOR LAB PRESS, 1 BUNGTOWN RD, PLAINVIEW, NY 11724.
ISSN: 0890-9369.
AU Patterson G I; Koweek A; Wong A; Liu Y X; **Ruvkun G (Reprint)**
AB Signals from TGF-beta superfamily receptors are transduced to the nucleus by Smad proteins, which transcriptionally activate target genes. In Caenorhabditis elegans, defects in a TGF-beta-related pathway cause a reversible developmental arrest and metabolic shift at the dauer larval stage. Null mutations in **daf**-3 suppress mutations in genes encoding this TGF-beta signal, its receptors, and associated Smad signal transduction proteins. **daf**-3 encodes a Smad protein that is most closely related to mammalian DPC4, and is expressed throughout development in many of the tissues that are remodeled during dauer development. **DAF**-4, the type II TGF-beta receptor in this pathway, is also expressed in remodeled tissues. These data suggest that the **DAF**-7 signal from sensory neurons acts as a neuroendocrine signal throughout the body to directly regulate developmental and metabolic shifts in tissues that are remodeled during dauer formation. A full-length functional **DAF**-3/GFP fusion protein is predominantly cytoplasmic, and this localization is independent of activity of the upstream TGF-beta-related pathway. However, this fusion protein is associated with chromosomes in mitotic cells, suggesting that **DAF**-3 binds DNA directly or indirectly. **DAF**-3 transgenes also interfere with dauer formation, perhaps attributable to a dosage effect. A truncated **DAF**-3/GFP fusion protein that is predominantly nuclear interferes with dauer formation, implying a role for **DAF**-3 in the nucleus. These data suggest that **DAF**-7 signal transduction antagonizes or modifies **DAF**-3 Smad activity in the nucleus to induce reproductive development; when **DAF**-7 signals are disabled, unmodified **DAF**-3 Smad activity mediates dauer arrest and its associated metabolic shift. Therefore, **daf**-3 is unique in that it is antagonized, rather than activated, by a TGF-beta pathway.

L3 ANSWER 7 OF 10 SCISEARCH COPYRIGHT 1999 ISI (R) DUPLICATE 5
TI The Fork head transcription factor **DAF**-16 transduces insulin-like metabolic and longevity signals in C-elegans
SO NATURE, (30 OCT 1997) Vol. 389, No. 6654, pp. 994-999.
Publisher: MACMILLAN MAGAZINES LTD, PORTERS SOUTH, 4 CRINAN ST, LONDON, ENGLAND N1 9XW.
ISSN: 0028-0836.

AU Ogg S; Paradis S; Gottlieb S; Patterson G I; Lee L; Tissenbaum H A; **Ruvkun G (Reprint)**

AB In mammals, insuli[redacted]ignalling regulates glucose transport [redacted]ether with the expression and activity of various metabolic enzymes. In the nematode Caenorhabditis elegans, a related pathway regulates metabolism, development and longevity(1,2). Wild-type animals enter the developmentally arrested dauer stage in response to high levels of a secreted pheromone(3), accumulating large amounts of fat in their intestines and hypodermis. Mutants in **DAF**-2 (a homologue of the mammalian insulin receptor) and AGE-1 (a homologue of the catalytic subunit of mammalian phosphatidylinositol 3-OH kinase) arrest development at the dauer stage(3), Moreover, animals bearing weak or temperature-sensitive mutations in **daf**-2 and age-1 can develop reproductively, but nevertheless show increased energy storage and longevity(1,2,4,5). Here we show that null mutations in **daf**-16 suppress the effects of mutations in **daf**-2 or age-1; lack of **daf**-16 bypasses the need for this insulin receptor-like signalling pathway. The principal role of **DAF**-2/AGE-1 signalling is thus to antagonize **DAF**-16. **daf**-16 is widely expressed and encodes three members of the Fork head family of transcription factors, The **DAF**-2 pathway acts synergistically with the pathway activated by a nematode TGF-beta-type signal, **DAF**-7, suggesting that **DAF**-16 cooperates with nematode SMAD proteins in regulating the transcription of key metabolic and developmental control genes. The probable human orthologues of **DAF**-16, FKHR and AFX, may also act downstream of insulin signalling and cooperate with TGF-beta effecters in mediating metabolic regulation. These genes may be dysregulated in diabetes.

L3 ANSWER 8 OF 10 SCISEARCH COPYRIGHT 1999 ISI (R) DUPLICATE 6

TI **daf**-2, an insulin receptor-like gene that regulates longevity and diapause in Caenorhabditis elegans

SO SCIENCE, (15 AUG 1997) Vol. 277, No. 5328, pp. 942-946.
Publisher: AMER ASSOC ADVANCEMENT SCIENCE, 1200 NEW YORK AVE, NW, WASHINGTON, DC 20005.
ISSN: 0036-8075.

AU Kimura K D; Tissenbaum H A; Liu Y X; **Ruvkun G (Reprint)**

AB A C. elegans neurosecretory signaling system regulates whether animals enter the reproductive life cycle or arrest development at the long-lived dauer diapause stage. **daf**-2, a key gene in the genetic pathway that mediates this endocrine signaling, encodes an insulin receptor family member. Decreases in **DAF**-2 signaling induce metabolic and developmental changes, as in mammalian metabolic control by the insulin receptor, Decreased **DAF**-2 signaling also causes an increase in life-span. Life-span regulation by insulin-like metabolic control is analogous to mammalian longevity enhancement induced by caloric restriction, suggesting a general link between metabolism, diapause, and longevity.

L3 ANSWER 9 OF 10 SCISEARCH COPYRIGHT 1999 ISI (R)

TI A PHOSPHATIDYLINOSITOL-3-OH KINASE FAMILY MEMBER REGULATING LONGEVITY AND DIAPAUSE IN CAENORHABDITIS-ELEGANS

SO NATURE, (08 AUG 1996) Vol. 382, No. 6591, pp. 536-539.
ISSN: 0028-0836.

AU MORRIS J Z; TISSENBAUM H A; **RUVKUN G (Reprint)**

AB A PHEROMONE-INDUCED neurosecretory pathway in Caenorhabditis elegans triggers developmental arrest and an increase in longevity at the dauer diapause stage. The gene age-1 is required for non-dauer development and normal senescence. age-1 encodes a homologue of mammalian phosphatidylinositol-3-OH kinase (PI(3)K) catalytic subunits. Lack of both maternal and zygotic age-1 activity causes dauer formation, whereas animals with maternal but not zygotic age-1 activity develop as non-dauers that live more than twice as long as normal. These data suggest that phosphatidylinositol signalling mediated by AGE-1 protein controls lifespan and the dauer diapause decision.

L3 ANSWER 10 OF 10 SCISEARCH COPYRIGHT 1999 ISI (R) DUPLICATE 7

TI **DAF**-2, **DAF**-16 AND **DAF**-23 - GENETICALLY INTERACTING GENES-CONTROLLING DAUER FORMATION IN CAENORHABDITIS-ELEGANS

SO GENETICS, (MAY 1994) Vol. 137, No. 1, pp. 107-120.
ISSN: 0016-6731.

AU GOTTLIEB S (Reprint); **RUVKUN G**

AB Under conditions of high population density and low food, Caenorhabditis elegans forms an alternative third larval stage, called the dauer stage, which is resistant to desiccation and harsh environments. Genetic analysis of some dauer constitutive (**Daf**-c) and dauer defective (**Daf**-d) mutants has revealed a complex pathway that is likely to function in particular neurons and/or responding tissues. Here we analyze the genetic interactions between three genes which comprise a branch of the dauer formation pathway that acts in parallel to or downstream of the other branches of the pathway, the **Daf**-c genes **daf**-2 and **daf**-23 and the **Daf**-d gene

daf-16. Unlike mutations in other **Daf**-c genes, mutations in both **daf**-2 and **daf**-23 cause non-conditional arrest at the dauer stage. Double mutant epistasis analysis suggests that **daf**-2 and **daf**-23 are functioning at a similar point in the dauer pathway. First, mutations in **daf**-2 and **daf**-23 are epistatic to mutations in the same set of **Daf**-d genes. Second, **daf**-2 and **daf**-23 mutants are suppressed by mutations in **daf**-16. Mutations in **daf**-16 do not suppress any of the other **Daf**-c mutants as efficiently as they suppress **daf**-2 and **daf**-23 mutants. Third, double mutants between either **daf**-2 or **daf**-23 and several other **daf**-d mutants exhibit an unusual interaction. Based on these results, we present a model for the function of **daf**-2, **daf**-23 and **daf**-16 in dauer formation.

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L1 116 S RUVKUN G/AU
L2 19 S L1 AND DAF
L3 10 DUP REM L2 (9 DUPLICATES REMOVED)
L4 1 S L3 AND PTEN
L5 3 S L1 AND PTEN
L6 1 DUP REM L5 (2 DUPLICATES REMOVED)

=> D L6

L6 ANSWER 1 OF 1 SCISEARCH COPYRIGHT 1999 ISI (R) DUPLICATE 1
AN 1999:53831 SCISEARCH
GA The Genuine Article (R) Number: 153WQ
TI The C-elegans **PTEN** homolog, DAF-18, acts in the insulin receptor-like metabolic signaling pathway
AU Ogg S; **Ruvkun G (Reprint)**
CS MASSACHUSETTS GEN HOSP, DEPT MOL BIOL, 50 BLOSSOM ST, BOSTON, MA 02114 (Reprint); MASSACHUSETTS GEN HOSP, DEPT MOL BIOL, BOSTON, MA 02114; HARVARD UNIV, SCH MED, DEPT GENET, BOSTON, MA 02115
CYA USA
SO MOLECULAR CELL, (DEC 1998) Vol. 2, No. 6, pp. 887-893.
Publisher: CELL PRESS, 1050 MASSACHUSETTES AVE, CIRCULATION DEPT, CAMBRIDGE, MA 02138.
ISSN: 1097-2765.
DT Article; Journal
FS LIFE
LA English
REC Reference Count: 36
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

=> D L5

L5 ANSWER 1 OF 3 SCISEARCH COPYRIGHT 1999 ISI (R)
AN 1999:53831 SCISEARCH
GA The Genuine Article (R) Number: 153WQ
TI The C-elegans **PTEN** homolog, DAF-18, acts in the insulin receptor-like metabolic signaling pathway
AU Ogg S; **Ruvkun G (Reprint)**
CS MASSACHUSETTS GEN HOSP, DEPT MOL BIOL, 50 BLOSSOM ST, BOSTON, MA 02114 (Reprint); MASSACHUSETTS GEN HOSP, DEPT MOL BIOL, BOSTON, MA 02114; HARVARD UNIV, SCH MED, DEPT GENET, BOSTON, MA 02115
CYA USA
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Publisher: CELL PRESS, 1050 MASSACHUSETTES AVE, CIRCULATION DEPT, CAMBRIDGE, MA 02138.
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L7 1 S (GLUCOSE TOLERANCE) AND DAF
L8 3 S OBESITY AND DAF
L9 2 DUP REM L8 (1 DUPLICATE REMOVED)

=> d Ti so au ab L7

L7 ANSWER 1 OF 1 CAPLUS COPYRIGHT 1999 ACS
TI Therapeutic and diagnostic tools for impaired **glucose tolerance** conditions based on the dauer polypeptides and genes of Caenorhabditis elegans
SO PCT Int. Appl., 202 pp.
CODEN: PIXXD2
IN Ruvkun, Gary; Kimura, Koutarou; Patterson, Garth; Ogg, Scott; Paradis, Suzanne; Tissenbaum, Heidi; Morris, Jason; Koweek, Allison; Pierce, Sarah
AB Disclosed herein are novel genes and methods for the screening of therapeutics useful for treating impaired **glucose tolerance** conditions, as well as diagnostics and therapeutic compns. for identifying or treating such conditions. The Caenorhabditis elegans metabolic regulatory genes **daf**-2 and age-1 encode homologs of the mammalian insulin receptor/phosphoinositide 3-kinase signaling pathway proteins, resp. In addn., the **DAF**-16 forkhead protein represents the major transcriptional output of this insulin signaling pathway. Dysregulation of the **DAF**-16 transcription factor in the absence of insulin signaling leads to metabolic defects; inactivation of **DAF**-16 reverses the metabolic defects caused by lack of insulin signaling in C. elegans. Finally, the C. elegans **daf**-7, da-1, **daf**-4, **daf**-8, **daf**-14, and **daf**-3 genes encode neuroendocrine/target tissue transforming growth factor-.beta. type signal transduction mols. that genetically interact with the insulin signaling pathway. Metabolic defects cause by lack of neuroendocrine TGF-.beta. signals can be reversed by inactivation of the **DAF**-3 transcription factor. The C. elegans **daf** genes are excellent candidate genes and proteins for human disease assocd. with glucose intolerance, e.g., diabetes, obesity, and atherosclerosis. The human homologs of these **daf** genes and proteins mediate insulin signaling in normal people and may be defective or mis-regulated in diabetics. Moreover, there are at least 2 classes of type II diabetics: those with defects in the TGF-.beta. signaling genes, and those with defects in insulin signaling genes. Exemplary sequences and functional characteristics are provided for the C. elegans **daf** homologs of the human genes: **daf**-2, **daf**-3 (3 differentially spliced isoforms), **daf**-16 (2 differentially spliced isoforms), age-1, and pdk-1 (two spliced isoforms).

=> d Ti so au ab L9 1-3

L9 ANSWER 1 OF 2 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 1
TI GLUT-4, tumor necrosis factor, essential fatty acids and **daf**-genes and their role in insulin resistance and non-insulin dependent diabetes mellitus
SO Prostaglandins, Leukotrienes Essent. Fatty Acids (1999), 60(1), 13-20
CODEN: PLEAEU; ISSN: 0952-3278
AU Das, U. N.
AB It is now believed that the GLUT-4 receptor, tumor necrosis factor-alpha (TNF-alpha), essential fatty acids (EFAs) and their metabolites and **daf**-genes have an important role in the development of **obesity** and non-insulin dependent diabetes mellitus (NIDDM). The protein encoded by **daf**-2 is 35% identical to the human insulin receptor, **daf**-7 codes a transforming growth factor-beta (TGF-beta) type signal and **daf**-16 can enhance superoxide dismutase (SOD) expression. EFAs and their metabolites can alter the cell membrane fluidity and enhance the expression of GLUT-4 and insulin receptors. EFAs can suppress TNF-alpha prodn. and secretion, a mechanism

that may have relevance to the role of these fatty acids in the pathogenesis of insulin resistance, **obesity** and NIDDM. Melatonin has anti-oxidant actions similar to **daf**-16, TGF-beta and SOD. Based on this evidence, it is proposed that GLUT-4, TNF-alpha, EFAs, **daf**-genes, melatonin and leptin interact with each other in ways which may have relevance to the development or abrogation of insulin resistance, **obesity**, NIDDM, complications due to NIDDM, longevity and ageing.

L9 ANSWER 2 OF 2 CAPLUS COPYRIGHT 1999 ACS

TI Therapeutic and diagnostic tools for impaired glucose tolerance conditions based on the dauer polypeptides and genes of Caenorhabditis elegans

SO PCT Int. Appl., 202 pp.
CODEN: PIXXD2

IN Ruvkun, Gary; Kimura, Koutarou; Patterson, Garth; Ogg, Scott; Paradis, Suzanne; Tissenbaum, Heidi; Morris, Jason; Koweek, Allison; Pierce, Sarah

AB Disclosed herein are novel genes and methods for the screening of therapeutics useful for treating impaired glucose tolerance conditions, as well as diagnostics and therapeutic compns. for identifying or treating such conditions. The Caenorhabditis elegans metabolic regulatory genes **daf**-2 and age-1 encode homologs of the mammalian insulin receptor/phosphoinositide 3-kinase signaling pathway proteins, resp. In addn., the **DAF**-16 forkhead protein represents the major transcriptional output of this insulin signaling pathway. Dysregulation of the **DAF**-16 transcription factor in the absence of insulin signaling leads to metabolic defects; inactivation of **DAF**-16 reverses the metabolic defects caused by lack of insulin signaling in C. elegans. Finally, the C. elegans **daf**-7, da-1, **daf**-4, **daf**-8, **daf**-14, and **daf**-3 genes encode neuroendocrine/target tissue transforming growth factor-.beta. type signal transduction mols. that genetically interact with the insulin signaling pathway. Metabolic defects cause by lack of neuroendocrine TGF-.beta. signals can be reversed by inactivation of the **DAF**-3 transcription factor. The C. elegans **daf** genes are excellent candidate genes and proteins for human disease assocd. with glucose intolerance, e.g., diabetes, **obesity**, and atherosclerosis. The human homologs of these **daf** genes and proteins mediate insulin signaling in normal people and may be defective or mis-regulated in diabetics. Moreover, there are at least 2 classes of type II diabetics: those with defects in the TGF-.beta. signaling genes, and those with defects in insulin signaling genes. Exemplary sequences and functional characteristics are provided for the C. elegans **daf** homologs of the human genes: **daf**-2, **daf**-3 (3 differentially spliced isoforms), **daf**-16 (2 differentially spliced isoforms), age-1, and pdk-1 (two spliced isoforms).

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L7 1 S (GLUCOSE TOLERANCE) AND DAF
L8 3 S OBESITY AND DAF
L9 2 DUP REM L8 (1 DUPLICATE REMOVED)
L10 952 S PTEN
L11 455 S L10 AND PHOSPHATASE
L12 8 S L11 AND DAF-18
L13 3 DUP REM L12 (5 DUPLICATES REMOVED)

=> d Ti so au ab L13 1-3

L13 ANSWER 1 OF 3 SCISEARCH COPYRIGHT 1999 ISI (R)
TI Regulation of the insulin-like developmental pathway of Caenorhabditis elegans by a homolog of the **PTEN** tumor suppressor gene
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (16 MAR 1999) Vol. 96, No. 6, pp. 2925-2930.
Publisher: NATL ACAD SCIENCES, 2101 CONSTITUTION AVE NW, WASHINGTON, DC 20418.
ISSN: 0027-8424.
AU Gil E B; Link E M; Liu L X; Johnson C D; Lees J A (Reprint)
AB The human **PTEN** tumor suppressor gene is mutated in a wide variety of sporadic tumors. To determine the function of **PTEN** in vivo we have studied a **PTEN** homolog in Caenorhabditis elegans, We have generated a strong loss-of function allele of the **PTEN** homolog and shown that the deficient strain is unable to enter dauer diapause, An insulin-like phosphatidylinositol 3-OH kinase (PI3'K) signaling pathway regulates dauer-stage entry. Mutations in either the daf-2 insulin receptor-like (IRL) gene or the age-1 encoded PI3'K catalytic subunit homolog cause constitutive dauer formation and also affect the life span, brood size, and metabolism of nondauer animals. Strikingly, loss-of-function mutations in the age-1 PI3'K and daf-2 IRL genes are suppressed by loss-of-function mutations in the **PTEN** homolog, me establish that the **PTEN** homolog is encoded by **daf-18**, a previously uncloned gene that has been shown to interact genetically with the DAF-2 IRL AGE-1 PI3'K signaling pathway, This interaction provides clear genetic evidence that **PTEN** acts to antagonize PI3'K function in vivo, Given the conservation of the PI3'K signaling pathway between C. elegans and mammals, the analysis of **daf-18** **PTEN** mutant nematodes should shed light on the role of human **PTEN** in the etiology of metabolic disease, aging, and cancer.

L13 ANSWER 2 OF 3 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 1
TI Regulation of dauer larva development in Caenorhabditis elegans by **daf-18**, a homolog of the tumor suppressor **PTEN**
SO Curr. Biol. (1999), 9(6), 329-332
CODEN: CUBLE2; ISSN: 0960-9822
AU Rouault, Jean-Pierre; Kuwabara, Patricia E.; Sinilnikova, Olga M.; Duret, Laurent; Thierry-Mieg, Danielle; Billaud, Marc
AB The tumor suppressor gene **PTEN** (also called MMAC1 or TEP1) is somatically mutated in a variety of cancer types [1-4]. In addn., germline mutation of **PTEN** is responsible for two dominantly inherited, related cancer syndromes called Cowden disease and Bannayan-Ruvalcaba-Riley syndrome [4]. **PTEN** encodes a dual-specificity **phosphatase** that inhibits cell spreading and migration partly by inhibiting integrin-mediated signalling [5-7]. Furthermore, **PTEN** regulates the levels of phosphatidylinositol 3,4,5-trisphosphate (PIP3) by specifically dephosphorylating position 3 on the inositol ring [8]. We report here that the dauer formation gene **daf-18** is the Caenorhabditis elegans homolog of **PTEN**. **DAF-18** is a component of the insulin-like signalling pathway controlling entry into diapause and adult longevity that is regulated by the DAF-2 receptor tyrosice kinase and the AGE-1 PI 3-kinase [9]. Others have shown that mutation of **daf-18** suppresses the life extension and constitutive dauer formation

assocd. with daf-2 or age-1 mutants. Similarly, we show that inactivation of **daf-18** by RNA-mediated interference mimics this suppression, and that wild-type **daf-18** transgene rescues the dauer defect. These results indicate that **PTEN**/**DAF-18** antagonizes the DAF-2-AGE-1 pathway, perhaps by catalyzing dephosphorylation of the PIP3 generated by AGE-1. These data further support the notion that mutations of **PTEN** contribute to the development of human neoplasia through an aberrant activation of the PI 3-kinase signalling cascade.

L13 ANSWER 3 OF 3 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 2

TI The C. elegans **PTEN** homolog, **DAF-18**, acts in the insulin receptor-like metabolic signaling pathway

SO Mol. Cell (1998), 2(6), 887-893
CODEN: MOCEFL; ISSN: 1097-2765

AU Ogg, Scott; Ruvkun, Gary

AB An insulin-like signaling pathway, from the DAF-2 receptor, the AGE-1 phosphoinositide 3-kinase, and the AKT-1/AKT-2 serine/threonine kinases to the DAF-16 Fork head transcription factor, regulates the metab., development, and life span of Caenorhabditis elegans. Inhibition of **daf-18** gene activity bypasses the normal requirement for AGE-1 and partially bypasses the need for DAF-2 signaling. The suppression of age-1 mutations by a **daf-18** mutation depends on AKT-1/AKT-2 signaling, showing that **DAF-18** acts between AGE-1 and the AKT input to DAF-16 transcriptional regulation. **Daf-18** encodes a homolog of the human tumor suppressor **PTEN** (MMAC1/TEP1), which has 3-**phosphatase** activity toward phosphatidylinositol 3,4,5-trisphosphate (PIP3). **DAF-18** **PTEN** may normally limit AKT-1 and AKT-2 activation by decreasing PIP3 levels. The action of **daf-18** in this metabolic control pathway suggests that mammalian **PTEN** may modulate insulin signaling and may be variant in diabetic pedigrees.

(FILE 'HOME' ENTERED AT 12:52:00 ON 21 MAY 1999)

FILE 'CAPLUS, SCISEARCH, MEDLINE, BIOSIS, CANCERLIT, AGRICOLA' ENTERED AT 12:52:04 ON 21 MAY 1999

```
L1          116 S RUVKUN G/AU
L2           19 S L1 AND DAF
L3           10 DUP REM L2 (9 DUPLICATES REMOVED)
L4            1 S L3 AND PTEN
L5            3 S L1 AND PTEN
L6            1 DUP REM L5 (2 DUPLICATES REMOVED)
L7            1 S (GLUCOSE TOLERANCE) AND DAF
L8            3 S OBESITY AND DAF
L9            2 DUP REM L8 (1 DUPLICATE REMOVED)
L10         952 S PTEN
L11         455 S L10 AND PHOSPHATASE
L12           8 S L11 AND DAF-18
L13           3 DUP REM L12 (5 DUPLICATES REMOVED)

=> S L11 AND ASSAY

L14          16 L11 AND ASSAY

=> DUP REM L14

PROCESSING COMPLETED FOR L14
L15           6 DUP REM L14 (10 DUPLICATES REMOVED)

=> d Ti so au ab L15 1-6
```

L15 ANSWER 1 OF 6 BIOSIS COPYRIGHT 1999 BIOSIS

TI The tumor suppressor, **PTEN**/MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3,4,5-trisphosphate.

SO Journal of Biological Chemistry, (May 29, 1998) Vol. 273, No. 22, pp. 13375-13378.
ISSN: 0021-9258.

AU Maehama, Tomohiko; Dixon, Jack E.

AB Phosphatidylinositol 3,4,5-trisphosphate (Ptd-Ins(3,4,5)P3) is a key molecule involved in cell growth signaling. We demonstrated that overexpression of **PTEN**, a putative tumor suppressor, reduced insulin-induced PtdIns(3,4,5)P3 production in human 293 cells without effecting insulin-induced phosphoinositide 3-kinase activation. Further, transfection of the catalytically inactive mutant of **PTEN** (C124S) caused PtdIns(3,4,5)P3 accumulation in the absence of insulin stimulation. Purified recombinant **PTEN** catalyzed dephosphorylation of PtdIns(3,4,5)P3, specifically at position 3 on the inositol ring. **PTEN** also exhibited 3-**phosphatase** activity toward inositol 1,3,4,5-tetrakisphosphate. Our results raise the possibility that **PTEN** acts in vivo as a phosphoinositide 3-**phosphatase** by regulating PtdIns(3,4,5)P3 levels. As expected, the C124S mutant of **PTEN** was incapable of catalyzing dephosphorylation of PtdIns(3,4,5)P3 consistent with the mechanism observed in protein-tyrosine **phosphatase**-catalyzed reactions.

L15 ANSWER 2 OF 6 BIOSIS COPYRIGHT 1999 BIOSIS

TI The C. elegans **PTEN** homolog, DAF-18, acts in the insulin receptor-like metabolic signaling pathway.

SO Molecular Cell, (Dec., 1998) Vol. 2, No. 6, pp. 887-893.
ISSN: 1097-2765.

AU Ogg, Scott; Ruvkun, Gary (1)

AB An insulin-like signaling pathway, from the DAF-2 receptor, the AGE-1 phosphoinositide 3-kinase, and the AKT-1/AKT-2 serine/threonine kinases to the DAF-16 Fork head transcription factor, regulates the metabolism, development, and life span of Caenorhabditis elegans. Inhibition of daf-18 gene activity bypasses the normal requirement for AGE-1 and partially bypasses the need for DAF-2 signaling. The suppression of age-1 mutations by a daf-18 mutation depends on AKT-1/AKT-2 signaling, showing that DAF-18 acts between AGE-1 and the AT input to DAF-16 transcriptional regulation, daf-18 encodes a homolog of the human tumor suppressor **PTEN** (MMAC1/TEP1), which had 3-**phosphatase** activity toward phosphatidylinositol 3,4,5-trisphosphate (PIP3). DAF-18 **PTEN** may normally limit AKT-1 and AKT-2 activation by decreasing PIP3 levels. The action of daf-18 in this metabolic control pathway suggests that mammalian **PTEN** may modulate insulin signaling and may be variant in diabetic

pedigrees.

L15 ANSWER 3 OF 6 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 1
TI Germline mutations in **PTEN** are an infrequent cause of genetic predisposition to breast cancer
SO Oncogene (1998), 17(6), 727-731
CODEN: ONCNES; ISSN: 0950-9232
AU FitzGerald, Michael G.; Marsh, Debbie J.; Wahrer, Doke; Bell, Daphne; Caron, Stacey; Shannon, Kristen E.; Ishioka, Chikashi; Isselbacher, Kurt J.; Garber, Judy E.; Eng, Charis; Haber, Daniel A.
AB Heterozygous germline mutations in **PTEN** are responsible for most cases of Cowden Syndrome, a rare familial trait characterized by hamartomas and by predisposition to cancer of the breast and thyroid. The variable and often subtle clin. findings that characterize Cowden Syndrome are frequently unrecognized, raising the possibility that germline **PTEN** mutations may confer susceptibility to breast cancer in women who have not been diagnosed with this syndrome. To det. whether such mutations contribute to genetic predisposition to breast cancer within the general population, the authors analyzed a cohort of women with early-onset breast cancer (<age 40), a subset of the population at increased risk for genetic susceptibility. Lymphoblast cell lines were analyzed using either direct nucleotide sequencing (28 cases), denaturing gradient gel electrophoresis (DGGE) (34 cases) or a yeast-based truncation **assay** (110 cases). No definitive, truncating mutations were obsd. in 172 patients. Missense changes were noted in the germline of 2/60 patients analyzed by direct nucleotide sequencing or DGGE, including a non-conservative amino acid substitution within the **phosphatase** domain, but neither showed loss of the wild-type allele in the corresponding breast tumor specimen. The authors conclude that germline mutations in **PTEN** are an uncommon cause of genetic predisposition to breast cancer within the general population.

L15 ANSWER 4 OF 6 MEDLINE DUPLICATE 2
TI Analysis of **PTEN**/MMAC1 alterations in aerodigestive tract tumors.
SO CANCER RESEARCH, (1998 Feb 1) 58 (3) 509-11.
Journal code: CNF. ISSN: 0008-5472.
AU Okami K; Wu L; Riggins G; Cairns P; Goggins M; Evron E; Halachmi N; Ahrendt S A; Reed A L; Hilgers W; Kern S E; Koch W M; Sidransky D; Jen J
AB **PTEN**/MMAC1 is a candidate tumor suppressor gene recently identified at chromosomal band 10q23. It is mutated in sporadic brain, breast, and prostate cancer and in the germ line of patients with hereditary Cowden disease. We searched for genetic alterations of the **PTEN**/MMAC1 gene in 39 primary head and neck cancers (HNSCCs), 42 primary non-small cell lung cancers (NSCLCs), 80 pancreatic cancer xenografts, and 37 cell lines and xenografts from colon, lung, and gastric cancers. Microsatellite analysis revealed loss of heterozygosity at markers near the gene in 41% of primary HNSCCs, 50% of NSCLCs, and 39% of the pancreatic cancers. Three cases of HNSCCs displayed homozygous deletion involving the gene. We sequenced the entire coding region of the **PTEN**/MMAC1 gene in the remaining tumors displaying loss of heterozygosity and found one terminating mutation in a HNSCC sample. Thus, a second inactivation event was observed in 4 of 39 primary HNSCC cases. By use of a protein truncation **assay**, one terminating mutation was also identified in one of eight NSCLC cell lines. Our results suggest that **PTEN**/MMAC1 gene inactivation plays a role in the genesis of some tumor types.

L15 ANSWER 5 OF 6 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 3
TI Growth suppression of glioma cells by **PTEN** requires a functional **phosphatase** catalytic domain
SO Proc. Natl. Acad. Sci. U. S. A. (1997), 94(23), 12479-12484
CODEN: PNASA6; ISSN: 0027-8424
AU Furnari, Frank B.; Lin, Hong; Su Huang, H. -J.; Cavenee, Webster K.
AB Deletions of all or part of chromosome 10 are the most common genetic alternations in high-grade gliomas. The **PTEN** gene (also called MMAC1 and TEP1) maps to chromosome region 10q23 and has been implicated as a target of alteration in gliomas and also in other cancers such as those of the breast, prostate, and kidney. Here, the authors sought to provide a functional test of its candidacy as a growth suppressor in glioma cells. The authors used a combination of Northern blot anal., protein truncation **assays**, and sequence anal. to det. the types and frequency of **PTEN** mutations in glioma cell lines so that the authors could define appropriate recipients to assess the growth suppressive function of **PTEN** by gene transfer. Introduction of wild-type **PTEN** into glioma cells contg. endogenous mutant alleles caused growth suppression, but was without effect in cells contg. endogenous wild-type **PTEN**. The ectopic expression of **PTEN** alleles, which carried mutations found in primary tumors and have been shown or are expected to inactivate its **phosphatase** activity, caused little growth suppression. Thus, **PTEN** is a protein **phosphatase** that exhibits functional and specific growth-suppressing activity.

L15 ANSWER 6 OF 6 MEDLINE DUPLICATE 4
TI **PTEN**/MMAC1 mutations [redacted] EGFR amplification in glioblastomas.
SO CANCER RESEARCH, (1997 Dec 1) 57 (23) 5254-7.
Journal code: CNF. ISSN: 0008-5472.
AU Liu W; James C D; Frederick L; Alderete B E; Jenkins R B
AB Loss of heterozygosity (LOH) from chromosome 10 is a hallmark of glioblastoma, the most malignant (grade IV) form of glioma. A candidate tumor suppressor gene, **PTEN**/MMAC1, that may be targeted for deletion in association with chromosome 10 LOH has recently been identified. Here we have investigated 63 glioblastomas for **PTEN** /MMAC1 alterations and identified DNA sequence changes that would affect the encoded protein in 17 (27%) tumors. Microsatellite analyses of normal-tumor DNA pairs were performed on 14 of these cases and revealed LOH at locations flanking and/or near **PTEN**/MMAC1 in all but 1 instance, suggesting that deletion of the remaining wild-type allele had occurred in the large majority of tumors with **PTEN**/MMAC1 mutations. Competitive PCR **assays** were developed to address the possible occurrence of **PTEN**/MMAC1 homozygous deletions in glioblastomas, and this analysis identified three samples having loss of both **PTEN**/MMAC1 alleles. EGFR amplification was determined to occur at similar frequencies among cases with or without **PTEN** /MMAC1 homozygous deletions or mutations, suggesting that a growth-promoting effect resulting from amplification-associated increases in epidermal growth factor receptor signaling is not necessarily dependent on the inactivation of **PTEN**/MMAC1.

1. **5,196,333**, Mar. 23, 1993, DNA sequences involved in neuronal degeneration, multicellular organisms containing same and uses thereof; Marin Chalfie, et al., 435/369, 29, 69.1, 70.3; 536/23.5 [IMAGE AVAILABLE]

US PAT NO: **5,196,333** [IMAGE AVAILABLE] L1: 1 of 1
DATE ISSUED: Mar. 23, 1993
TITLE: DNA sequences involved in neuronal degeneration, multicellular organisms containing same and uses thereof
INVENTOR: Marin Chalfie, New York, NY
Eve Wolinsky, Princeton, NJ
Monica Driscoll, New York, NY
ASSIGNEE: The Trustees of Columbia University, New York, NY (U.S. corp.)
APPL-NO: 07/530,968
DATE FILED: May 30, 1990
ART-UNIT: 184
PRIM-EXMR: Robert A. Wax
ASST-EXMR: Miguel Escallon
LEGAL-REP: John P. White

US PAT NO: **5,196,333** [IMAGE AVAILABLE] L1: 1 of 1

ABSTRACT:
This invention provides an isolated nucleic acid molecule encoding a wild-type animal protein associated with neuronal degeneration and an isolated nucleic acid molecule encoding a mutated animal protein associated with neuronal degeneration. Also provided are strains of the nematode Caenorhabditis elegans containing the nucleic acid molecules encoding a mutated C. elegans protein associated with neuronal degeneration. The invention also provides methods for detecting such nucleic acid molecules, for diagnosing degenerative disease, for causing a diseased human cell to degenerate, and for screening drugs to identify drugs which prevent or decrease neuronal degeneration.

CLAIMS:

CLMS(1)

What is claimed is:

1. An isolated nucleic acid molecule encoding a wild-type C. elegans protein, wherein the C. elegans protein is encoded by the deg-1 gene which has the DNA sequence shown in FIG. 7 and, when mutated, is the genetic basis of neuronal degeneration associated with a neurodegenerative disorder.

CLMS(2)

2. An isolated nucleic acid molecule encoding a mutated C. elegans protein, wherein the mutated C. elegans protein is encoded by a mutated deg-1 gene which forms the genetic basis of neuronal degeneration associated with a neurodegenerative disorder.

CLMS(3)

3. A Caenorhabditis elegans strain containing an isolated nucleic acid molecule encoding a mutated C. elegans protein, wherein the mutated C. elegans protein is encoded by a mutant of the deg-1 gene designated u38, the deg-1 gene having the cDNA sequence shown in FIG. 7, with said strain designated TU38 and deposited with the ATCC under Accession No. 40818.

CLMS(4)

4. A Caenorhabditis elegans strain containing an isolated nucleic acid molecule encoding a mutated C. elegans protein, wherein the mutated C. elegans protein is encoded by a mutant of the deg-1 gene designated uIn1, the deg-1 gene having the cDNA sequence shown in FIG. 7, with said strain designated TU1191, and deposited with the ATCC under Accession No. 40817.

CLMS(5)

5. A Caenorhabditis elegans strain containing an isolated nucleic acid

molecule encoding a mutated C. elegans protein, wherein the mutated C. elegans protein is encoded by a mutant of the mec-4 gene designated e1611, the mec-4 gene having the cDNA sequence shown in FIG. 9, with said strain designated CB1611, and deposited with the ATCC under Accession No. 40820.

CLMS(6)

6. A Caenorhabditis elegans strain containing an isolated nucleic acid molecule encoding a mutated C. elegans protein, wherein the mutated C. elegans protein is encoded by a mutant of the mec-4 gene designated u214, the mec-4 gene having the cDNA sequence shown in FIG. 9, with said strain designated TU214 and deposited with the ATCC under Accession No. 40819.

CLMS(7)

7. A Caenorhabditis elegans strain containing an isolated nucleic acid molecule encoding a mutated C. elegans protein, wherein the mutated C. elegans protein is encoded by a mutant of the mec-4 gene designated u231, the mec-4 gene having the cDNA sequence shown in FIG. 9, with said strain designated TU231 and deposited with the ATCC under Accession No. 40821.